

BACTERIA OF *BACILLUS CEREUS* GROUP IN CEREALS AT RETAIL

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The subject of analysis were three main types of cereals available at retail on the local market. The objective was to assess the extent of cereals contamination by *B. cereus* group and to indicate the species dominant in cereals contamination.

Bacteria of *B. cereus* group were present in 89.6% of the retail packs tested. The isolation frequency of *B. cereus* from pearl barley, buckwheat and wheat cereals was 85%, 85.7% and 100%, respectively, with contamination level, in majority of the analysed samples (60.4%), exceeding 10^2 MPN/g.

The strains prevailing in cereals contamination did not hydrolyse starch and were β hemolytic. Out of the 380 isolated strains, those not hydrolysing starch accounted for 56%, with only 16 strains (4.2%) not able both to hydrolyse starch and non-hemolytic. Amongst the strains growing at 4° to 30° C the ability to hydrolyse starch and to perform β hemolysis – the features attributed to diarrheal strains – was expressed by 36.9% strains. Twenty strains (5.3% of the total number) – non-hemolytic, unable to hydrolyse starch and not growing at 4° C – were considered as potentially emetic ones.

Mesophilic, lecithinase-positive strains, β hemolysis-positive, unable to hydrolyse starch predominant in the tested cereals (208/380) were identified with the API 50 CHB tests (Bio Merieux) as *B. cereus* type 1. *B. cereus* type 1 was the prevailing contaminant in barley and wheat cereals. In buckwheat, the most commonly isolated species was *B. mycoides*.

INTRODUCTION

By cereals we mean whole, hulled, cracked, rolled or ground forms of products produced from various kinds of grains, most frequently deprived of inassimilable ingredients [Świdorski, 1999]. Manufactured from barley, oat, whey, buckwheat, millet, corn, rice, etc. at retail they are offered, usually, as whole grain cereals (buckwheat), hulled cereals (millet), grits (barley, wheat), flakes (oats) or meals (corn, semolina). As the group of products rich in fiber, vitamins and minerals, cereals constitute valuable components of a diet.

The production of cereals in Poland exceeds 75000 tons per year. An average Pole consumes about 3 kg of cereals annually [Anonymous 1, 2007]. The price and tradition are usually decisive factors in the selection of the type of grains and form of cereals by consumers.

Products of plant origin, including cereals, can be the carrier of various types of microflora, including moulds, yeast, bacteria belonging to *Enterobacteriaceae* family, *Pseudomonas* spp., *Clostridium perfringens*, *Bacillus cereus*, *Bacillus sphaericus*, coagulase-positive staphylococci or aerobic mesophilic bacteria [Aziz *et al.*, 2006].

In different countries, quality control of plant products, including grains, is based on various quality indicators and different criteria set for the acceptable contamination level to be met [IMNRC, 2003; EC Regulation No. 2073/2005].

One of the criteria commonly used in microbiological quality assessment of that group of products is the total viable count (TVC) of mesophilic bacteria, with the acceptable

level of contamination varying significantly due to the subject of the study and the country [IMNRC, 2003]. For cereals, e.g. in Spain or Cuba, the acceptable TVC accounts for $<10^4$ cfu/g, whilst for rice and its products in The Netherlands – for $<10^6$ cfu/g.

The *Bacillus cereus* group, as a separate microbiological criterion in the quality assessment of grains and grain products is taken into account, e.g. in Ireland, Spain or The Netherlands. In Spain the acceptable contamination of cereals with *B. cereus* is $<10^1$ cfu/g, whereas in The Netherlands it accounts for 10^1 cfu of *B. cereus* per 1 gram whilst in Ireland – for $<10^2$ cfu of *B. cereus* and *B. subtilis* per 1 g in rice [IMNRC, 2003].

Polish regulations addressing to quality assessment of grain products [PN-V-74003:2004; PN-A-74205:1997; PN-V-74004:2004] are based on organoleptic (taste after cooking, odour, colour) and physicochemical analyses, e.g. moisture content, organic contaminants contents, presence of metals, glass and other inorganic particles, presence of mites and pests and their remainders, granulation, ash content, as well as transportation and storage conditions.

Even though in Poland no criteria have been stipulated for microbiological quality assessment of cereals, their nutritional values and growing popularity among the health-aware population rises the question about potential health hazard to consumers posed by spore-forming bacteria of the *B. cereus* group that might be the grain/cereals contaminants.

The objective of the study was, therefore, to determine whether and to what extent the cereals available on the local

market are contaminated with bacteria from *B. cereus* group, and which species of this group are the predominant ones.

MATERIALS AND METHODS

The subject of analysis were 3 types of cereals available at retail market in one of the supermarkets in Szczecin. Between October 2006 and March 2007 the total number of 48 separate packets of cereals, including 5 after the expiry date declared by the producer, were tested.

Two 10-g subsamples were collected from each retail packet and initial ten-fold dilutions were prepared in buffered 0.1% peptone water in sterile flasks with glass beads. After 5 min of shaking in a WU-4 shaker (Premed) the flasks were subjected to heating at 80°C for 10 min (± 2 min) in a water bath and then quickly cooled under tap running cold water.

The total count of *B. cereus* group spores in 1 g of cereals was determined with the MPN (Most Probable Number) method [PN-EN ISO 21871: 2007], with the method's detectability defined as 1 cfu/g. Once diluted, each sub-sample was transferred into TSB (Tryptone Soya Broth – Oxoid) with polymyxin B in three repetitions. The inoculated TSB medium with polymyxin B, was then incubated at 30°C for 24-48 h. Growth-positive tubes were subcultured onto PEMBA medium (Oxoid) to separate colonies. The dishes were incubated at 30°C for 24-48 h. The dishes regarded as a positive result were those with colonies turquoise in colour with opaque zone around them, whereas a questionable result was indicated by colonies with fatty acids precipitation zone but characterised by untypical colour or *vice versa*.

For the identification, 1-3 colonies were selected at random. Their purity was tested by parallel subculturing to separate colonies on MYP Agar (Oxoid) and TSA (Tryptone Soya Agar – Oxoid) and incubation of plates at 30° for 24-48 h.

The isolated strains were then subjected to tests on: hemolysis (including its type) on TSA medium with ram's blood, starch hydrolysis on nutrient agar with starch, colony

morphology on TSA medium and ability to grow and the type of growth (pellicle, diffusive, sediment) on TSB medium at 4°C (14 days), 30°C and 42°C (48 h), respectively.

The selected strains representing particular types of growth and biochemical activity were identified using API 50 CHB tests (BioMerieux).

The differences in frequency rate and counts of *B. cereus* in the types of cereals subjected to analysis were calculated using the χ^2 method, and noted significant at $p \leq 0.05$.

RESULTS

Results of the microbiological analysis of cereals purchased at the local market indicated that bacteria from *B. cereus* group were present in 89.6% (43/48) of the analysed samples (Table 1). Among the 3 types of grains subjected to analysis only the wheat cereals were positive in 100%. In the case of barley and buckwheat, the *B. cereus*-positive retail packets accounted for 85% (17/20) and 85.7% (12/14), respectively. A range in the frequency rate of *B. cereus*-positive samples between the types of grains tested was slightly broader, oscillating between 66.7 and 100%. However, the observed differences in the occurrence of *B. cereus* group due to the form of grains tested turned out to be statistically insignificant.

The lack of relevant quantitative criteria for microbiological quality assessment of cereals in Polish regulations was the cause that the results of quantitative analyses were related to analogous criteria used in microbiological quality assessment of rice in Ireland [IMNRC, 2003]. According to those guidelines, the acceptable level of contamination with bacteria from *B. cereus* and *B. subtilis* group should be lower than 10^2 cfu/g. On that basis it was stated that only 39.5% (19/48) of the cereals samples tested met such quantitative criteria. In majority of the samples – 60.4% (29/48) – the contamination with *B. cereus* group exceeded 10^2 MPN/g. That type of contamination referred to 55% (11/20) of barley, 50% (7/14) of buckwheat and 78.6% (11/14) of wheat cereals

TABLE 1. Isolation frequency and contamination level of selected cereals with *Bacillus cereus* group.

Type of grains	Type of cereals	Samples (no.)	Positive samples	Contamination level (MPN/g)		
				1* – $<10^1$	$\geq 10^1$ – $<10^2$	$>10^2$
Barley	wiejska	6	4	2	-	2
	pearl	6	6	2	-	4
	with vegetables	4	3	1	-	2
	hulled	4	4	-	1	3
Σ		20	17 (85%)	5	1	11
Buckwheat	roasted	12	10	2	2	6
	with vegetables	2	2	-	1	1
Σ		14	12 (85.7%)	2	3	7
Wheat	hulled	2	2	-	-	2
	grits (kuskus)	8	8	-	-	8
	semolina	4	4	1	2	1
Σ		14	14 (100%)	1	2	11
Σ		48	43 (89.6%)	8	6	29

* detection limit of the method applied

TABLE 2. Type of cereal grains versus strains ability for blood hemolysis and starch hydrolysis.

Type of grains	Strains (no.)	Colonies (PEMBA medium)		Blood hemolysis (β)		Starch hydrolysis	
		typical	atypical	+	-	+	-
Barley	157	151		134	17	61/12	73/5
			6	4	2	3/1	1/1
Buckwheat	76	73		60	13	19/8	40/6
			3	2	1	2	1
Wheat	147	122		112	10	28/5	84/5
			25	13	12	6/1	7/11
Σ	380	346		306	40	108/25	197/16
			34	19	15	9/2	10/13

samples tested. The differences observed were, however, also statistically insignificant.

The comparison of the share of single packaging of cereals with acceptable and unacceptable levels of contamination with bacteria belonging to *B. cereus* group, including classification into types of cereals, showed statistically significant ($p < 0.01$) differences only in the case of the types of wheat cereals (whole grain wheat cereals, kus kus, semolina). The level of contamination with *B. cereus* in semolina was significantly lower than in the two other types of wheat cereals.

Although among the colonies growing on PEMBA medium there predominated the typical ones: turquoise in colour and lecithinase-positive, except for the typical ones also colonies having only one of the typical features – definitely less numerous ones, were isolated for further analysis.

In total, 380 strains were subjected to a phenotypic analysis, out of which 346 (91.1%) were recognised as typical for the analysed group of bacteria, based on the type of growth on PEMBA medium (Table 2). Among the “typical” representatives of *B. cereus* group, the majority (306/346) performed type β hemolysis, out of which only 35% (108/306) had the ability to hydrolyse starch. Worthy of notice are quantitative relations between the strains able to hydrolyse starch (S+) and those not having this ability (S-), (Table 2). With predomination of the strains unable to hydrolyse starch (S-) and performing β hemolysis ($\beta+$), in all types of cereals, the number of S- strains was three times (wheat cereals) or twice (buckwheat cereals) higher than the number of S+ strains. Only in the case of pearled barley was the number of S+ and S- strains within the number of “typical” strains giving β hemolysis ($\beta+$) similar (Table 2).

Typical strains, not hydrolysing starch, accounted for 56% (213/380) of all the strains isolated, including 16 ones (4.2%) which showed neither the ability to hydrolyse starch nor to perform β hemolysis.

Among 34 “questionable” strains the majority was lacking both the ability to hydrolyse starch and to perform β hemolysis (S-, β -; 13/15) or, was lacking the ability to hydrolyse starch but performed β hemolysis (S-, $\beta+$; 10/19). The strains classified as “atypical” were isolated mainly from wheat cereals (Table 2).

One of the factors taken into consideration in differentiation of the strains, appointed initially to *B. cereus* group,

were the differences in colony morphology on TSA medium after 48 h of incubation at 30°C. On the basis of differences in morphology there were nine types of colonies selected, differing in shape, edge, structure, elevation, size and colour. Predominant colonies were large, flat, over 1 cm in diameter, with irregular edges, whitish, rarely white – greyish or brownish. Rhizoid type of growth was characteristic only for 3 out of 157 colonies isolated from barley cereals. However, the strains identified as *B. mycoides* not always display the rhizoid type of growth. It is quite likely for that type of strains to represent *B. pseudomycoides*, the species absent on the list of the API 50CHB identification tests (BioMerieux).

Data concerning differences in colony, cells and endospores morphology in bacteria from *B. cereus* group are to be presented elsewhere.

The differences in temperature requirements of the strains isolated from the cereals were determined after incubation of the material collected from pure, 24-h colony on TSA medium and subcultured into TSB medium at temperatures of 4°C; 30°C and 42°C for 14 days and 48 h, respectively. The analysis demonstrated differences in the presence and type of growth of the strains tested with 7 types of growth noted, *i.e.* diffusive one (d), pellicle (p), sediment (s), pellicle and sediment (p+s), pellicle and diffusion (p+d), diffusion and sediment (d+s) and pellicle, diffusion and sediment (p+d+s) (Table 3).

At 30°C, the temperature optimal for the tested group of bacteria, all the strains yielded growth, with the predominating type of growth after 48-h incubation at 30°C being pellicle + sediment (161/380). The most infrequent – a diffusive type of growth, was characteristic for 2 strains, only (Table 3).

The growth in the form of pellicle + sediment was observed most often (157/359) also at the temperature of 42°C. At this temperature, diffusion was only a part of the type of growth second in frequency (147/359) described as pellicle + diffusion + sediment (Table 3).

As a result of 14-day incubation of the strains at 4°C, “the growth” was noted for 111 out of 380 tested strains. After the incubation, the presence of sediment was observed in majority of cases (78/111), which does not signify the growth of the strain at this temperature but is a result of the transformation of vegetative forms suspended in the medium into spores, forming sediment on the bottom of a tube. Growing

TABLE 3. Effect of temperature on growth ability and type of growth of strains isolated from cereals.

Type of growth* (n=380)	Incubation temperature (°C)		
	4	30	42
p	5	67	29
d	2	2	-
s	78	9	14
p+s	4	161	157
p+d	-	27	8
d+s	15	22	4
p+d+s	7	92	147
Σ	111	380	359

*Type of growth: p = pellicle; d = diffusive one; s = sediment; p+s = pellicle and sediment; p+d = pellicle and diffusive one; d+s = diffusive and sediment; p+d+s = pellicle, diffusive one and sediment.

abilities at 4°C can be attributed to the remaining 33 strains displaying other types of growth (Table 3).

The differences in the type of growth of the tested strains at selected temperatures could be due to species diversity as well as an “age” differentiation of individuals within the sub-cultured material.

Interesting information was obtained when comparing data concerning ranges of temperatures at which the strains isolated from cereals were observed to grow (Table 4). In all of the cereals tested, the highest percentage was reported for strains able to grow at 30-42°C (255/380). Fourteen strains yielded growth only at 30°C. Among 111 strains “able to grow” at 4°C only 7 did not grow at 42°C (Table 3 and 4). Although among the *B. cereus* representatives isolated from cereals there predominated strains mesophilic in nature, a significant number of psychrotrophic strains (104/380) able to grow in a temperature range of 4-42°C – i.e. including cold storage temperatures – needs to be emphasized. Growth abilities of the strains from *B. cereus* group under cold storage might, when refrigerating meals including cereals, lead to particular health problems.

Data concerning the relation between the presence of features connected with pathogenicity of the strains from *B. cereus* group and the range of their growth temperatures are presented in Table 5. Among the strains “growing” within the range of 4-30°C the ability to hydrolyse starch and perform β hemolysis – the features ascribed to diarrhoeal strains, were demonstrated by 36.9% (41/111) of the strains. Twenty

TABLE 4. Abilities of strains isolated from cereals for growth at the tested temperature ranges.

Type of cereals	Numbers of strains giving growth at particular temperature range (°C)			
	4-30	30	30-42	4-42
Barley (n=157)	51	6	100	46
Buckwheat (n=76)	19	4	53	18
Wheat (n=147)	41	4	102	40
Σ (n=380)	111	14	255	104

out of the strains isolated from cereals (5.3% of all strains tested), which gave weak hemolysis or were unable to perform it, did not hydrolyse starch and did not grow at 4°C, could be regarded as potentially *B. cereus* emetic strains. Among the isolated strains the largest group was constituted by the strains performing β hemolysis and not hydrolysing starch (208/380; Table 5).

Strains with a particular type of growth and biochemical activity, selected at random, were subjected to the identification using API 50 CHB tests (BioMerieux). The biochemical profiles of the strains thus obtained showed *B. cereus* type 1 and *B. mycoides* to be species predominating in contamination of the cereals analysed (Table 6).

With results of the identification of the tested strains being accepted as “good” or “very good”, the percent of conformity of the features to the pattern for strains identified as *B. cereus* type 1 was in the range from 42.7% (very good identification) to 92.5% (very good identification) and for strains identified as *B. mycoides* in the range of 53.5–97.9% (good identification) (Table 6).

For strains identified as *B. cereus* type 1, the feature considered as atypical was a lack of ability to ferment D-ribose, only. It should be noted that very good identification to *B. cereus* type 1 was reported for the strains isolated from wheat cereals both these hydrolysing and unable to hydrolyse starch. In the case of *B. mycoides*, features recognized as atypical for the species were the ability to decompose glycerol (2/4 strains), ferment glucose (3/4 strains) and a lack of ability to ferment D-mannose (3/4 strains).

The results obtained showed that in contamination of wheat and barley cereals the predominating species from *B. cereus* group was *B. cereus* type 1. In the case of buckwheat cereals the most commonly isolated species was *B. mycoides*.

DISCUSSION

Although cereals, likewise rice, belong to the group of grain products more and more commonly consumed also in Poland, available literature provides sparse and somehow outdated information about cereals as a carrier of *B. cereus* responsible for foodborne diseases [Goepfert et al., 1972; Haque & Russell, 2005; Johnson, 1984; Norris et al., 1981; Sarrias et al., 2002; Thorsen et al., 2006]. *Bacillus cereus* is currently one of the six subspecies within the *B. cereus* group including *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. anthracis*, *B. weihen-*

TABLE 5. Relation between the presence of features relevant to *B. cereus* pathogenicity and growth ability of the strains within specified temperature ranges.

Ability for: blood hemolysis of β type (β) and starch hydrolysis (S) (n=380)	Temperature (°C)			
	4-30	30	30-42	4-42
β + S + (n=118)	41	5	72	36
β – S – (n=28)	8	0	20	7
β – S + (n=26)	12	3	11	11
β + S – (n=208)	50	6	152	50
Σ	111	14	255	104

TABLE 6. Identification of strains isolated from cereals to the species level based on API 50CHB (bioMerieux).

Identified species	Type of cereals	Type β hemolysis	Starch hydrolysis	Features compatibility (%) API 50CHB	Identification to species level	No. of strains with similar features
<i>B.cereus</i> 1	buckwheat	+	+	92.5	good	3
<i>B.mycoides</i> / <i>B.cereus</i> 1	buckwheat	+	+	64.7 / 34.7	good	16
<i>B.mycoides</i> / <i>B.cereus</i> 1	buckwheat	+	+	69.0 / 30.3	good	
<i>B.cereus</i> 1	wheat	+	+	84.8	very good	2
<i>B.mycoides</i> / <i>B.cereus</i> 1	wheat	+	+	53.5 / 43.1	good	11
<i>B.mycoides</i>	wheat	+	+	97.9	good	21
<i>B.cereus</i> 1 / <i>B.mycoides</i>	wheat	+	-	42.7 / 29.7	very good	16
<i>B.cereus</i> 1	wheat	+	-	91.3	very good	93

stephanensis and *B. pseudomycooides* [Nakamura, 1998]. These bacteria isolated with various frequency from food poisoning cases in humans (diarrhoeal and emetic strains) might also be the causative agents of e.g. eye infections, meningitis or bacteremia [Drobniewski, 1993].

As an integral component of soil microflora [Norris *et al.*, 1981], the spores of bacteria from *B. cereus* group can easily contaminate plant raw materials, such as grain products, including cereals.

The microbiological analysis of commercially offered single packagings of 3 types of cereals confirmed a high frequency rate of bacteria from *B. cereus* group in these types of foodstuffs. The bacteria contaminated from 85% (barley cereals) to 100% (wheat cereals) of the cereal retail packagings under study.

In majority of the cereals analysed (60.4%), the level of contamination with bacteria belonging to *B. cereus* group exceeded 10^2 MPN/g.

With a large variety of species representing the genus *Bacillus* it is essential to separate those pathogenic to humans. Among the features mentioned as useful in such a differentiation there is ability to produce lecithinase, to perform β hemolysis and to hydrolyse starch. Although, according to Holbrook & Anderson [1980], lecithinase production is considered to be one of the typical features, not all the bacteria from *B. cereus* group are capable of it. Due to Hendriksen *et al.* [2005], species included into *B. cereus* group differ in their ability to decompose lecithin, from good reported for *B. cereus* and *B. weihenstephanensis* to weak demonstrated for *B. mycooides*.

In the reported study, all the strains with typical colour of colony on selective PEMBA medium, isolated from cereals, were lecithinase-positive (91.1%). Moreover, some strains with atypical colour of the colony on the medium also displayed that feature. Three strains with the rhizoid type of growth, typical of *B. mycooides*, were characterised by little ability to decompose lecithin and ability to produce hemolysin.

When differentiating within the genus *Bacillus*, apart from the test for lecithinase determinations involve also the ability to hydrolyse starch [Trojanowska *et al.*, 1996]. According to Pirhonen *et al.* [2005], a test for hydrolysis of starch enables differentiating between emetic and diarrhoeal strains. As the authors stated, emetic strains produce lecithinase, do not hydrolyse starch and most often give weak hemolysis re-

action. According to Zaremba & Borowski [2001], numerous clinical strains (diarrhoeal), except for *B. anthracis*, perform β hemolysis. Moreover, all the strains giving a wide zone of hemolysis on blood agar decomposed starch and lecithin. According to Hendriksen *et al.* [2005], the representatives of *B. cereus* group differ in their hemolytic activity with hemolytic activity of *B. cereus* being significantly lower than the one performed by *B. weihenstephanensis* and *B. mycooides* species. Yet some of the emetic *B. weihenstephanensis* strains (MC67 and MC118) able to hydrolyse starch were shown to be non-hemolytic [Thorsen *et al.*, 2006].

In our own studies, in each of the types of cereals examined the predominating strains did not hydrolyse starch and performed β hemolysis. Typical strains, not hydrolysing starch, constituted 56% of all isolated strains, including only 16 (4.2%) which did not hydrolyse starch and did not show blood hemolysis.

With the temperature of 30°C preferred by *B. cereus* group for growth, the strains tested differed in their ability and type of growth in the liquid medium at 4°C/14 days and at 30°C and 42°C/48 h.

According to reference data, the maximum temperature for the growth of *B. cereus* group is 50°C or 55°C [Trojanowska *et al.*, 1996]. When it comes to the minimal temperatures, they are in the range of 10-12°C, or even 4-6°C [Carlin *et al.*, 2006]. For instance, *B. mycooides* does not grow at 6°C and *B. weihenstephanensis* – at 42°C [Hendriksen *et al.*, 2005; Lechner *et al.*, 1998]. According to Carlin *et al.* [2006], the growth of emetic strains is feasible within the range from 10°C to 48°C.

In the reported study, 6 out of 32 strains growing at 4°C did not grow at 42°C. It may indicate that these strains belong to *B. weihenstephanensis*.

For aerobic/facultatively anaerobic representatives of *B. cereus* group, a diffusion with a slight sediment, and for some species also pellicle on the surface of the liquid medium, are considered a typical type of growth [Trojanowska *et al.*, 1996].

Among the tested strains incubated at 30°C and 42°C/48 h the predominating type of growth was pellicle + sediment (Table 3). After 14 days of incubation of the cultures at 4°C, in 20.5% of the strains the presence of sediment was observed on the bottom of the tube, which was formed most likely by sedimenting spores produced by vegetative cells introduced initially into the medium.

Among the strains growing within the temperature range from 4°C to 30°C the ability to hydrolyse starch and perform β hemolysis (features ascribed to diarrhoeal strains) was observed in 36.9% of the strains. Twenty strains (5.3% of the total number) hemolysis-negative, unable to hydrolyse starch and not growing at 4°C can be recognised as potentially emetic *B. cereus* strains. Also Altayar & Sutherland [2006] showed a low percentage of emetic strains in the total number of bacteria from *B. cereus* group. Among the strains they examined, only 3.2% were emetic *B. cereus*. Nevertheless, while discussing the emetic strains of *B. cereus* group worthy of remembering are the psychrotrophic non-hemolytic, hydrolysing starch *B. weihenstephanensis* strains MC67 and MC118 [Thorsen et al., 2006] or β hemolysis- and starch hydrolysis-positive emetic *B. cereus* reference strain F4810/72 (DSM 4312) [data not published].

In the cereals analysed, the predominating group was constituted by mesophilic strains able to perform β hemolysis and not hydrolysing starch (208/380), which on the basis of API 50 CHB (BioMerieux) tests were identified as *B. cereus* type 1. The results of the biochemical identification pointed at *B. cereus* type and *B. mycoides* as species prevailing in contamination of the tested cereals (Table 6). In contamination of wheat and barley cereals the predominating species was *B. cereus* type 1. In buckwheat cereals the most commonly isolated species was *B. mycoides*.

The predomination of *B. cereus* type 1 in contamination of wheat cereals, combined with determined frequency of contamination (100% of samples) and the number of *B. cereus* in 1 g of this type of cereals should be a warning to the common consumers of this type of cereals who belong to YOPI risk groups (Young, Old, Pregnant, Immunocompromised).

According to the results obtained, the differentiation to the species level within *B. cereus* group, based on the tests determining the ability of a strain to produce lecithinase, hydrolyse starch, hemolyse blood, differences in morphology of colonies, abilities to grow under various temperature conditions and also using ready identification tests (considering the lack of reading data for species representing *B. cereus* group, such as *B. thuringiensis*, *B. weihenstephanensis* and *B. pseudomycoides*) must be regarded as highly imperfect. Additional difficulty in the identification into species is posed by phenotypic variability of bacteria from *B. cereus* group.

Further research planned, aimed at identify the genes responsible for cereulide toxin production (emetic strains), will hopefully enable to determine to what extent the presence/absence of the ability to produce lecithinase, blood hemolysis and/or starch hydrolysis can help to distinguish emetic *B. cereus* strains.

CONCLUSIONS

1. Cereals available at retail market in Poland are common carriers of bacteria from *B. cereus* group.

2. In majority of retail cereals packagings the level of contamination with *B. cereus* group exceeded 10^2 MPN/g.

3. The bacteria of *B. cereus* group predominating in cereals contamination were lecithinase-positive, β hemolytic strains, unable to hydrolyse starch.

4. Potentially emetic strains of *B. cereus* constituted 5.3% of all strains isolated from cereals.

5. In contamination of wheat and barley cereals the predominating species was *B. cereus* type 1, with *B. mycoides* predominating in buckwheat cereals.

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